On the Variation in the Growth of Mammalian Tissue in Vitro *according to the Age of the Animal.*

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[Plate 15.]

In a previous communication* it was shown that there was considerable variation in the value, as a culture medium, of plasmata taken from different animals of the same species; that these plasmata did not vary as to whether they were homogenous or autogenous, but that some plasmata were good media and some were bad. During this investigation certain evidence arose that this difference might in part be due to the age of the animal.

In the present investigation a series of experiments was carried out to show what was the effect, if any, of the age of the animal upon the plasma as a culture medium, and upon the tissues as regards the power of growth. Carrel, Burrows, Harrison, and Ingebrigtsen have shown in several papers that embryonic tissue tends to grow more rapidly and more vigorously than adult tissue. There appears to have been, however, no work conducted on the characters of the plasma, although it has been frequently assumed that the plasma of the young or embryonic animals makes a more suitable medium than that of adults; nevertheless it was permissible to believe that the reverse might in fact be true, and that the plasma of young animals is a less suitable medium. It would appear important that this point should be settled, that thereby evidence might be gained as to the controlling influences on the growth of young tissue *in vivo*.

Technique.

The following experiments were carried out entirely with the tissues of rabbits. As far as possible animals were used that had been bred in the laboratory, so that the exact age was known. This was the case with all the young animals. In certain cases, however, adult rabbits were bought of unknown age, but in such cases they were all fully grown and therefore could be used as adult animals. As far as could be judged, they were all over a year old. The technique of Carrel was rigorously adhered to, the tissues being grown in pure plasma so that the characters of the growth might be

* 'Roy. Soc. Proc.,' B, vol. 87, p. 452 (1914).

unaffected by the presence of any stimulating or inhibiting substance. In the majority of cases two tissues were used, so as to lessen, as far as possible, any experimental errors. A few cultures were made of the spleen, but most of the experiments were carried out with thyroid and liver. These tissues as a rule grow well, and the growth is not obscured by the emigration of cells, as so often happens when spleen is used.

Young testicular tissue was not cultivated in the present series of experiments, for it was considered that, as this tissue only fully develops later in life, false conclusions might be arrived at if the immature testicle of the young rabbit was used. In the majority of cases fresh tissue was made use of, and in this case cross experiments were generally performed, the tissue of young and old animals being grown in plasmata of both animals. A certain number of experiments were conducted with stock cultures of adult testicle which had been growing for ten generations in a medium of plasma and tissue extract.

(1) *Cultivations of Splenic Tissue*—

Experiment 1.—An adult rabbit two years old was anæsthetised. The fur on the ventral surface of the body was removed, the skin sterilised, the carotid artery exposed, and the blood collected in sterile paraffined tubes placed in ice. The blood was then collected from a young animal 10 days old and placed in ice-cold paraffined tubes. Both bloods were centrifugalised.

The spleen was removed from the young and old animals and placed in Ringer's fluid. Four cultures were made of each spleen in each plasma, so that there were four groups.

At the end of 12 hours there was good emigration of round cells in all the preparations, but it was more marked in the case of the spleen of the young animals both in the young and old plasmata. At the end of 48 hours there was a well marked growth of retiform tissue, which formed mosaic-like masses in the case of the young spleen in the old plasma, but was present in a less marked degree in the case of the old spleen in the old plasma. In both cases such growth was apparently absent when the young plasma was used.

Owing to the amount of round cell emigration it was difficult to estimate accurately the extent of the growth, and hence experiments with this tissue were discontinued.

(2) *Cultivation of Thyroid and Liver Tissues*—

Eleven experiments, comprising 282 cultivations, were carried out in this group. In all cases both the thyroid and liver were cultivated at the same time. By this means experimental errors were less likely, for if the results

were due to such errors they would be less likely to occur in both groups. The ages of the young animals varied from two days to two weeks, and during this limited period there seemed to be little, if any, variation in the nature of the tissues and plasmata as regards the capacity for growth. One experiment will be described in detail, the others being carried out on precisely the same lines.

Experiment 2.—An adult rabbit over a year old was anæsthetised. The fur on the ventral surface of the body was removed, the skin sterilised, the carotid artery laid bare, and the blood collected in sterile paraffined tubes kept in ice. This animal was kept anæsthetised. Blood was collected from a young animal five days old and also placed in iced paraffined tubes. Its thyroid and a piece of liver were removed and placed in sterile Ringer's fluid. The young animal was killed. Similar tissues were removed from the old animal. The blood was centrifugalised. Cultures of the young animal were made in both plasmata, as were also those of the old animal, six cultures being made in each group.

The nature of the growth was observed and at certain periods specimens were fixed and stained. After 48 hours the thyroid of the young animal showed marked growth in the old plasma, but that of the old animal in the old plasma, although showing considerable growth both of the connective and parenchymatous types of cell, was definitely less than that of the young animal. On the other hand, both specimens in the young plasma showed either no growth at all or only a few cells growing from the edge of the tissue. Similar results were obtained in the case of the liver.

The above results are well shown by the following Table:—

	Old plasma.	Young plasma.
Old tissue	Moderate growth	Very slight growth.
Young tissue ...	Very good growth	Slight growth.

The above experiment was performed again 10 times. The old animals were in all cases fully developed, and the majority were known to be over one year of age. The young animals varied in age between two days and two weeks. The cultures were made in groups of four or six in each media and were prepared under identical conditions. By taking groups of four or six specimens it was possible to estimate more accurately the changes in growth, for in the primary cultures it is unusual, excepting perhaps in the case of the testicle, to find that all the specimens have grown to an identical extent. Moreover, the percentage of successful growths in a series is in

itself evidence of value of the suitability of the media and the power of the tissue to grow. The results of these experiments may be summarised as follows:—

(a) *Young Tissues Growing in Old Plasma.*—It was found that growth was in all cases extensive and successful. Cultivations occurred in 100 per cent. of the cases.

With the *thyroid,* masses of cuboidal cells were seen extending into the plasma, and between them were large numbers of branching connective tissue cells.

In the case of the *liver,* the growth was also extensive, and after 48 hours large masses of the characteristic deeply staining cuboidal cells were visible together with large numbers of connective tissue cells (fig. 1). Successful results were again obtained in 100 per cent. of the cases. In both groups the growth continued for four or five days before any signs of degeneration occurred.

(b) *Young Tissues Growing in Young Plasma.*—With this group there was a very marked difference. There is considerable difficulty in obtaining the blood of these young animals, owing to their small size, and it was at first thought possible that the difference in growth might be due to the fact that the plasma so obtained was not in good condition. It was found, however, that the results were practically constant even after these difficulties had been overcome.

In the case of the *thyroid* only 8 per cent. of the specimens showed any growth, and they were thus sharply differentiated from the specimens of the same tissue growing in old plasma. Even in those cases in which the growth was present it was slight in amount. In no case were any cuboidal cells seen, and even after three or four days there were only present a few connective cells growing from scattered areas at the edge of the tissue. This result was constant apart from liquefaction of the plasma, which in other cases has been found to limit growth. That is to say, the decrease in growth did not appear to be due to mechanical causes.

With the *liver* similar results were obtained. In this case a larger number of the preparations showed growth, the results being positive in 26 per cent. of the cases. In one specimen (Experiment 5) there was very good growth, but in three other specimens of the same series there was no growth, and such a result did not occur again in the other series. It is probable, therefore, that this result was an experimental error. In the remaining specimens which showed growth the extent of the growth was slight. In some cases a few outgrowths of cuboidal deeply staining cells were seen, but in no case were they so extensive as when old plasma was used as a medium.

In the majority of cases only a few branching connective tissue cells were visible, and they extended only for a short distance into the surrounding plasma, the difference between the amount of connective tissue growth in young and old plasma being very marked (fig. 2).

(c) *Old Tissues Growing in Old Plasma.*—In this group there was a moderate amount of growth, about 80 per cent. of the cultivations being successful. In every experiment some of the specimens showed good growth. On comparison, however, it was always seen that this growth was less than when young tissues were used.

In the case of the *thyroid* a few cuboidal cells were seen growing from the edge of the tissue after about 48 hours, growth being present in 70 per cent. of the specimens. After a further 24 hours connective tissue cells were visible. It was found in the parallel series that not only was growth more extensive when young tissue was used both as regards the parenchymatous and connective tissue cells, but that a larger percentage of specimens showed activity.

With the *liver* good results were obtained, the characters of the growth corresponding with that described in previous communications.* In this series growth took place in 88 per cent. of the specimens, and when present there were always to be seen masses of deeply staining cuboidal cells, but these masses were always less marked than when young tissue was grown in the same plasma. Proliferation of connective tissue cells took place at a slightly later date, the cells soon growing beyond and between the masses of parenchymatous cells. Here again the zone of connective tissue growth was always less marked than was the case with young tissue (fig. 3).

(d) *Old Tissue Growing in Young Plasma.*—This was found to be the worst combination. The tissues apparently were not so active and the medium was less suitable. In the majority of cases no growth took place. The tissues stained poorly and apparently died.

In the case of the *thyroid* only 3 per cent. of the specimens showed any growth at all, and even in the successful cases this was extremely slight. In the majority, even at the end of three or four days, the edge of the tissue remained sharply cut. In some the plasma was liquefied, but in others this change had not taken place, so that here again the absence of the growth was not dependent upon a mechanical factor. Even in the few cases where there was any evidence of growth, no cuboidal cells could be seen. At most there were one or two elongated connective tissue cells to be found after a careful search, so that at first sight there was a tendency to believe that no growth had taken place.

* 'The Journal of Pathology and Bacteriology,' vol. 18, p. 319 (1914).

With the *liver* there were positive results in 9 per cent. of the cases, but even in these growth was extremely slight. In no case were any cuboidal or parenchymatous cells seen. There was very slight growth of connective tissue, so that after three days there could be seen in a few cases several long connective tissue cells growing here and there from the edge of the tissue (fig. 4). Often a portion of the original tissue stained poorly and was manifestly dead.

These experiments, therefore, strongly confirmed the observations of previous workers, namely, that the tissue of young or embryonic animals shows more active growth than similar tissues taken from adult animals. In addition to this, it appeared manifest that the plasma of such young animals was not nearly so suitable a medium as the plasma of adult animals. Such a condition has not previously been described, and is so opposed to what one would at first sight believe, that it seemed necessary to confirm these experiments. For this purpose further experiments were carried out with stock cultures of rabbit testicle. These tissues had been growing *in vitro* for 10 generations in a medium composed of two parts of plasma and one part of spleen extract. At this period they were growing vigorously, so that at the end of 48 hours there was a wide zone of newly formed cells surrounding the original tissue.

Experiment 13.—Stock specimens of rabbit testicles as described above were cultivated in groups of four each—(*a*) in plasma obtained from a young animal 10 days old; (*b*) in plasma obtained from an adult rabbit over a year old; (*c*) in a mixture of plasma from an old animal, two parts, and spleen extract one part. At the end of 48 hours, the specimens cultivated in the mixture of old plasma and spleen extract were growing vigorously and showed a very wide zone of active cells, mainly of the connective tissue type (see fig. 5). These specimens served as a control. Those growing in old plasma also showed a wide zone of cells of the connective tissue type, but the newly formed cells, as was to be expected, owing to the absence of a stimulating extract, were considerably fewer in number and did not extend so far into the surrounding media (see fig. 6).

In the case of the tissues growing in young plasma, growth was very slight. There were only a few cells growing from the edge of the tissue (see fig. 7) and in these cells but few mitotic figures were seen.

This experiment confirmed those carried out with fresh tissues, and there can be no doubt that young plasma is a less suitable medium for the growth of tissues than that of old animals. It has been previously shown* that with older animals some plasmata are not such good media as others, and

* 'Roy. Soc. Proc.,' B, vol. 87, p. 452 (1914).

that in such cases the plasma is always improved if it be frozen for a period of about three days, which is very suggestive that the poor growth is due to the presence of an inhibiting substance. It is probable, therefore, that in young animals, also, the inhibiting substance is present in larger quantities, and hence the plasma makes a poor medium.

Conclusions.

1. Growth of tissues *in vitro* affords a valuable means of investigation as to the effects of age upon growth.

2. The tissues of young animals grow more rapidly and vigorously than those of adult animals.

3. The plasma of young animals is a much less suitable medium for the growth of tissue *in vitro* than the plasma of old animals.

4. The unsuitability of the plasma of young animals as a medium is probably due to the presence of an increased amount of some inhibiting substance.

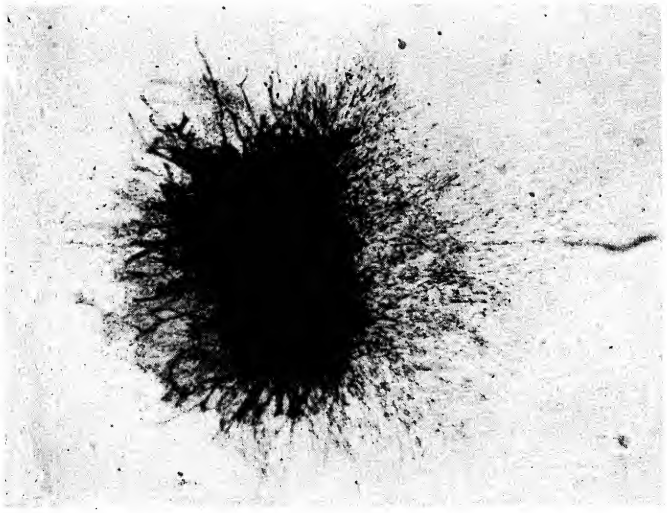
EXPLANATION OF PLATE.

Growth of Fresh Liver.

Fig. 1.—Two days' growth of young liver in old plasma.
Fig. 2.—Two days' growth of young liver in young plasma.
Fig. 3.—Two days' growth of old liver in old plasma.
Fig. 4.—Two days' growth of old liver in young plasma.

Growth of Stock Testicle.

Fig. 5.—Two days' growth of testicle in plasma plus spleen extract.
Fig. 6.—Two days' growth of testicle in old plasma.
Fig. 7.—Two days' growth of testicle in young plasma.



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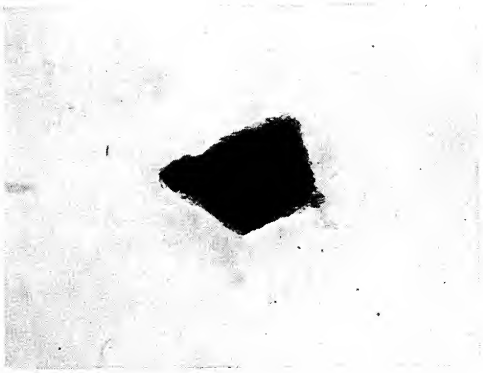

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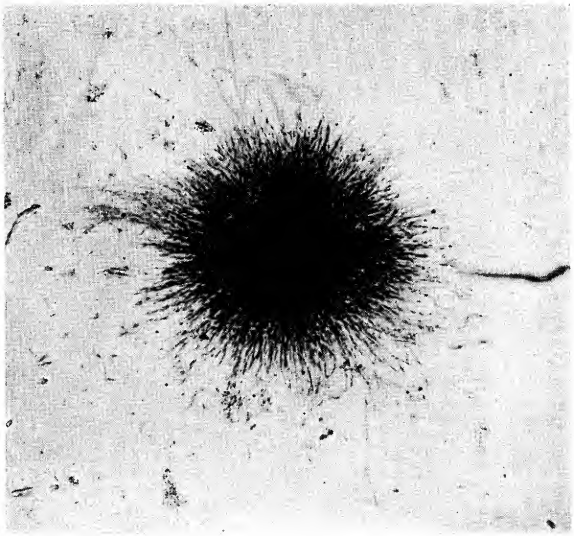

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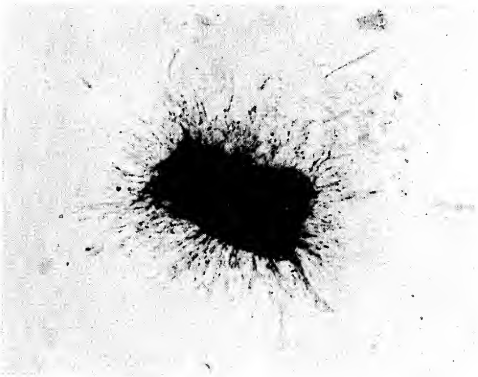

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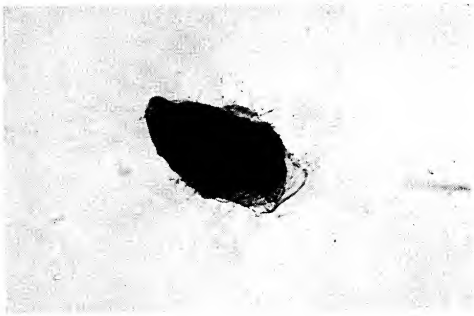

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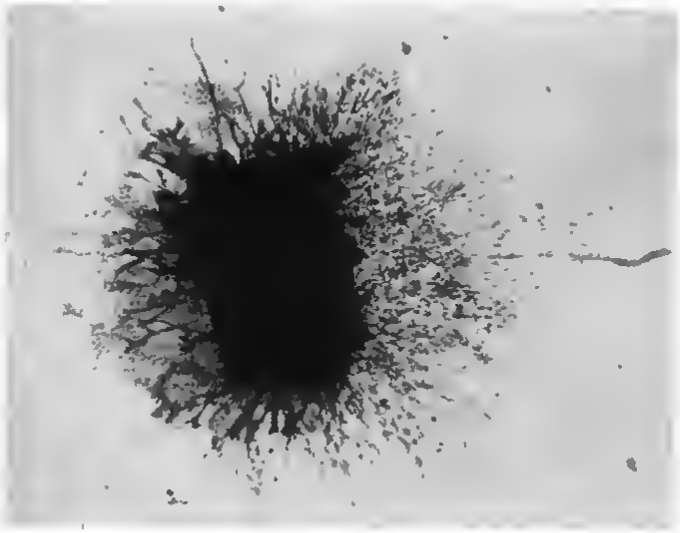

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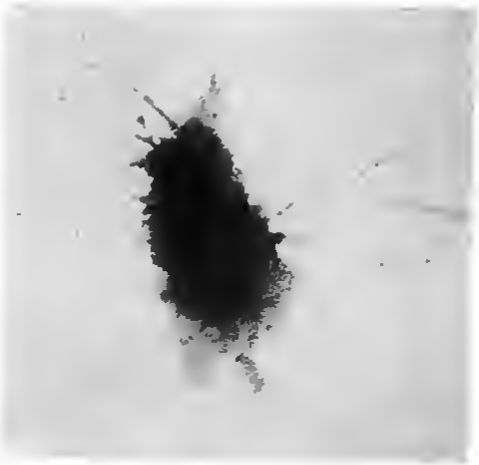

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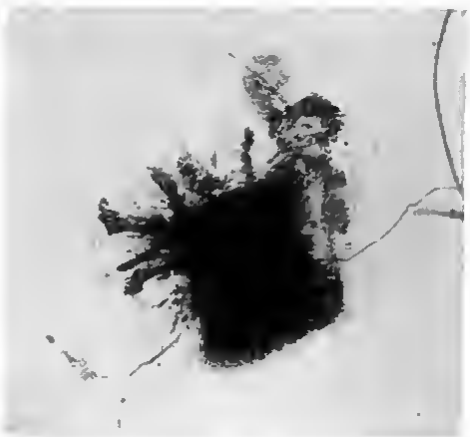

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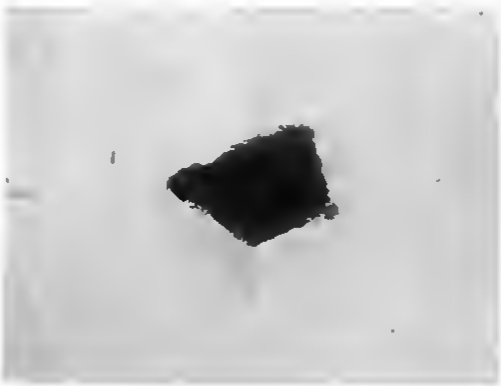

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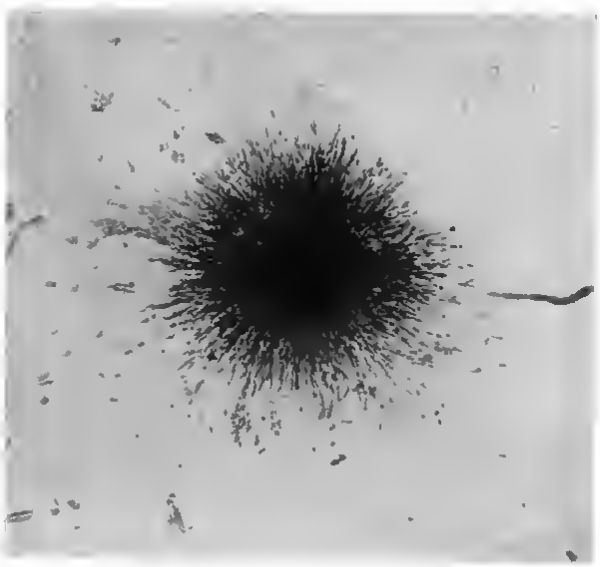

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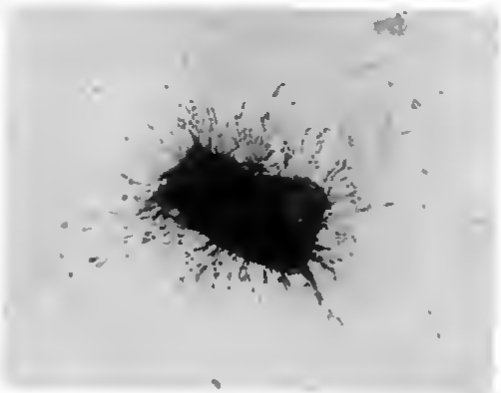

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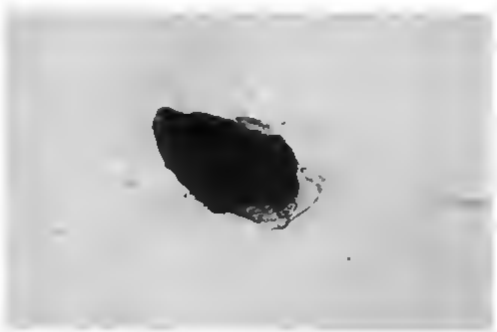

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